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Bark-Inhabiting Green Algae in Japan (2)
Chlorella ellipsoidea and *Ch. luteoviridis* (Chlorelloideae, Chlorellaceae, Chlorococcales)

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Two species of bark-inhabiting *Chlorella* were collected from the Uchibo area, Chiba Prefecture, Japan. One is ellipsoidal in shape and the other is spherical. The ellipsoidal alga has band- or saucer-shaped chloroplast and the pyrenoid is traversed by thylakoids. Pyrenoglobuli are aligned on both sides of the thylakoids. The spherical alga is relatively large and the chloroplast is mainly band-shaped. The thylakoids do not completely traverse the pyrenoid. Pyrenoglobuli are distributed around the thylakoids and often scattered throughout the pyrenoid matrix. These two algae are identified as *Ch. ellipsoidea* Gerneck and *Ch. luteoviridis* Chodat, respectively.

Introduction

In the course of our studies of bark-inhabiting green algae, several species of *Chlorella* and related algae were collected from the Uchibo area, Chiba Prefecture, Japan. Although many species of *Chlorella* were described to occur in aerial habitats, most identifications have been made only on the basis of gross morphology.

In the taxonomic studies of *Chlorella*, Shihira and Krauss (1965) examined the physiological and biochemical properties using culture specimens. Fott and Nováková (1969) carried out the taxonomic studies of 9 species with 6 varieties of *Chlorella* mainly based on the morphological features. Later, Kessler (1976, 1978, 1985a, 1985b, 1986, 1987) and Kessler and Huss (1992) studied the physiological and biochemical characters of *Chlorella* species in detail. Takeda (1988a, 1988b, 1991, 1993) showed that cell wall sugar composition could be used as a taxonomical marker for the classification of *Chlorella*. Recently, Ikeda and Takeda (1995) demonstrated the differences of pyrenoid structure among *Chlorella* species.

We have carried out a taxonomic study of the bark-inhabiting *Chlorella* and related algae with light and electron microscopy using laboratory cultured specimens. In this paper, two species, *Ch. ellipsoidea* Gerneck and *Ch. luteoviridis* Chodat are treated.

Materials and Methods

Specimens of *Chlorella ellipsoidea* and *Ch. luteoviridis* were collected from the bark of the palm *Trachycarpus fortunei* (Hooker) Wendland and *Pinus* *thunbergii* Parlatore, respectively, at Kaneda-kaigan Beach, Kisarazu City, on 5 June, 1993. Culture strains for comparison were obtained from Center for Cellular and Molecular Research at the University of Tokyo, formerly the Institute of Applied Microbiology (IAM), and Culture Collection of Algae at the University of Texas (UTEX). The algae examined in this study were cultured in Bold's basal medium (Bold 1949). The methods of unialgal culture and the procedure of transmission electron microscopy were obtained in a previous paper (Hanagata et al. 1996).

The strains examined are maintained in Chiba Laboratory, Mitsui Engineering & Shipbuilding Co., Ltd.

Results and Discussion

Chlorella ellipsoidea Gerneck

Light microscopy (Figs. 1, a–g, 2. a, b): The alga is unicellular and solitary. Cells are ellipsoidal, ovoid, 2.0–8.0 × 3.0–14 μm in size or rarely spherical, 5.0–12 μm in diameter. The chloroplast in young cells is parietal and saucer- or band-shaped, simple, with an smooth margin, filling more than a half of the cell lumen. In mature cells the chloroplast is parietal and irregularly undulated, sometimes possessing incisions. The pyrenoid is surrounded by several starch grains. Oil droplets are sometimes present in cytoplasm. Vacuoles are sometimes present in old cells. The cells produce 2–8 autospores. Reproduction takes place by the division of protoplast into polyhedric parts, with bluntly pointed ends, equal in size. Autospores are ellipsoidal to ovoid, 2.0–4.0 × 3.0–7.0 μm in size. The parent cell wall dissolves at one place and forms an aperture through which the autospores are discharged.

Electron microscopy (Fig. 2. c, d): The cell wall is single, 0.07–0.12 μm in thickness, and lacks electron-dense materials. The chloroplast consists of thylakoid lamellae arranged in several almost parallel rows. The pyrenoid matrix located near the center of the chloroplast is surrounded by several starch grains and traversed by the thylakoids. Many small pyrenoglobuli are arranged along the thylakoids. Autospores have numerous lipoidal globuli and several large particles which are well stained with OsO_4.

Comparison was made with the authentic culture strain, *Ch. ellipsoidea* IAM C-87. The cellular morphology and ultrastructure of our *Ch. ellipsoidea* was fundamentally identical with those of culture strain IAM C-87 (Fig. 4a), a relative of the neotype (SAG 211-1a). It is confusing whether *Ch. ellipsoidea* is merely a variety of *Ch. saccharophila* or an autonomous species. *Ch. saccharophila* was first described as *Chlorothecium saccharophilium* by Krüger (1894) on the basis of a specimen obtained from the sap of *Populus alba*. The species was later transferred to the genus *Chlorella* by Migula (1907). While *Ch. ellipsoidea* was first described by Gerneck (1907) on the basis of a specimen collected from a small pool. Fott and Nováková (1969) compared 13 strains of ellipsoidal *Chlorella* labeled either as *Ch. saccharophila* and *Ch. ellipsoidea*, and concluded that their morphological differences were only the shape of chloroplast and the presence or absence of starch grains. They considered that these differences were insufficient to establish them as separate species, since the morphology of chloroplast and starch grains easily varied depending on the culture conditions. They, therefore, treated both taxa merely as varieties. Kessler (1987) and Kessler and Huss (1992), however, demonstrated the different biochemical and physiological attributes of both taxa; growth on mannitol, cadmium sensitivity, suitabilities of mass culture in acid and salt water. They concluded that *Ch. ellipsoidea* should be regarded as an autonomous species rather than as a variety of *Ch. saccharophila*. Takeda (1993) reported that the stainability of the cell wall with ruthenium red and the anisotropy showed different patterns in these two species, although the rigid cell wall was mainly composed of the same sugars (glucose and mannose) in both taxa. Ikeda and

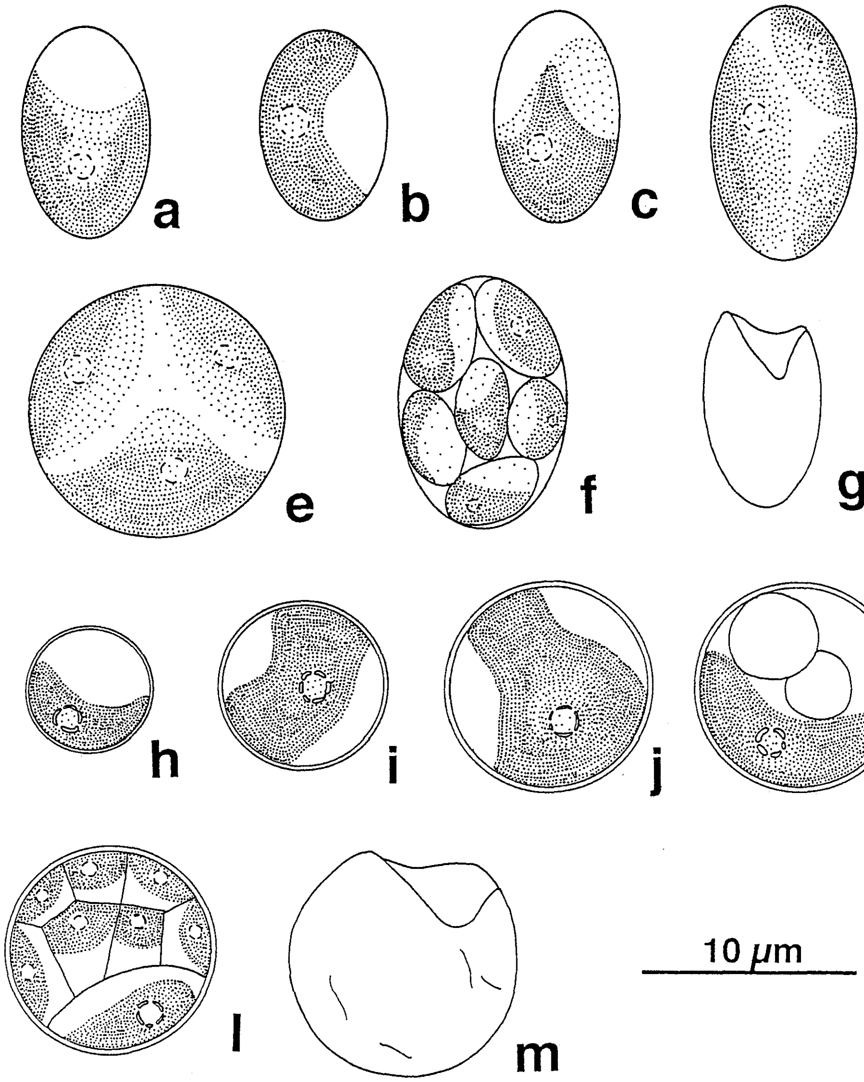

Fig. 1. a–g. *Chlorella ellipsoidea*. a–c. Vegetative cells. d, e. Division of the chloroplast. f. Autospores in the parent cell. g. Empty parent cell wall. h–m. *Chlorella luteoviridis*. h–k. Vegetative cells. I. Autospores in parent cell. m. Empty parent cell wall.

Takeda (1995) observed the pyrenoid structures of both taxa, and showed the pyrenoid structure of *Ch. ellipsoidea* was quite different from that of *Ch. saccharophila*. The recognition of these two algae as distinct taxa has also been supported by DNA/DNA reassociation study (Huss and Kessler 1987). In this study, *Ch. ellipsoidea* is treated as an autonomous species different from *Ch. saccharophila*.

According to Punčochářová (1994), culture strain (CAP A 76b labeled "*Ch. ellipsoidea*") observed by Fott and Nováková (1969) was not *Ch. ellipsoidea*, and she determined it as a new species, *Ch. trebouxioides*.

Chlorella luteoviridis Chodat

Light microscopy (Figs. 1. h–k, 3. a, b): The alga is

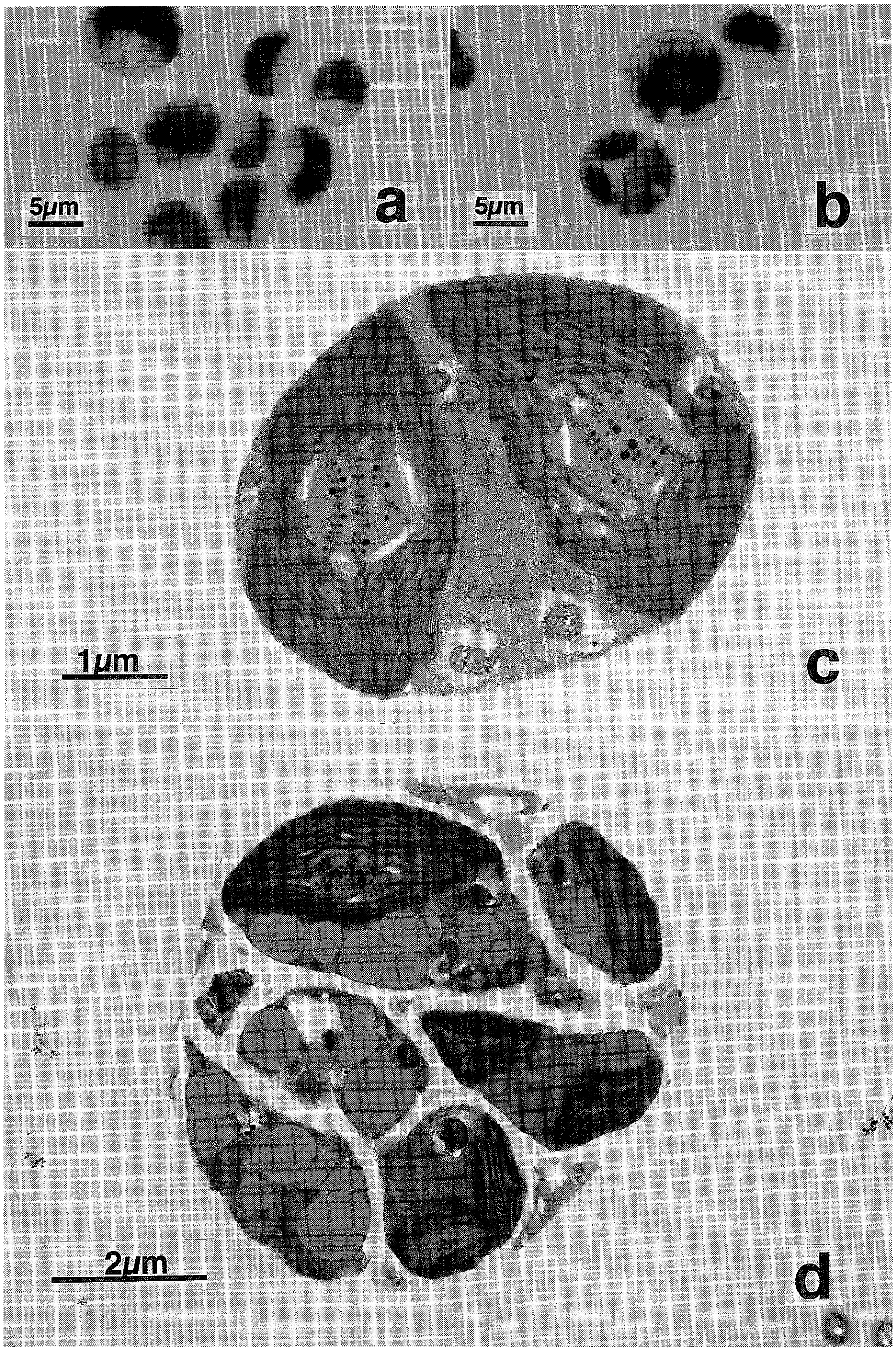

Fig. 2. *Chlorella ellipsoidea*. (a, b. Photomicrographs. c, d. Transmission electron micrographs.) a. Vegetative cells. b. Sporangium. c. Section of vegetative cell, showing distribution of major organelles and pyrenoid morphology. d. Section of sporangium, showing formation of autospores.

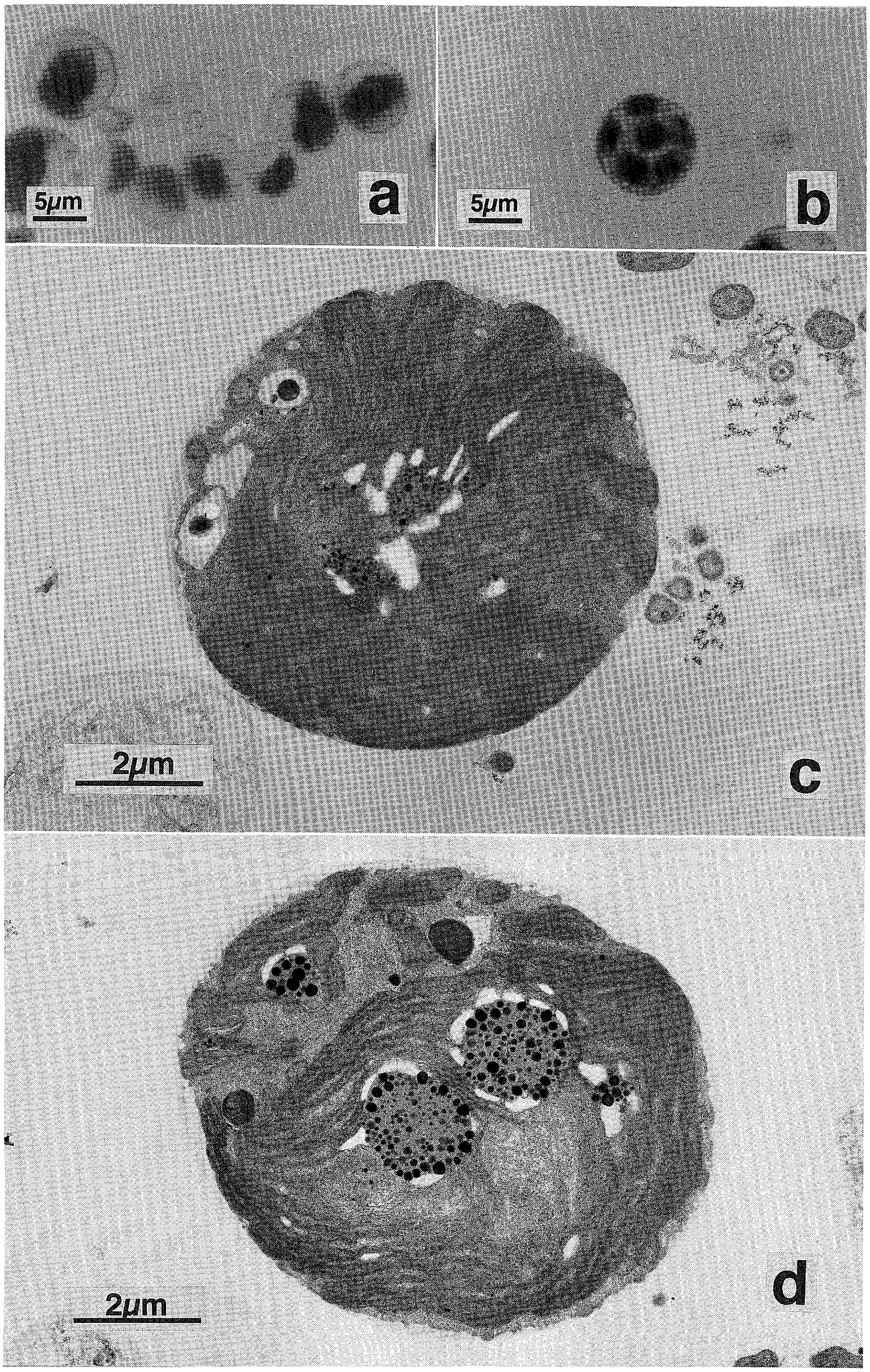

Fig. 3. *Chlorella luteoviridis*. (a, b. Photomicrographs. c, d. Transmission electron micrographs.) a. Vegetative cells. b. Sporangium. c. Section of vegetative cell, showing small pyrenoglobuli aligned both sides of the thylakoids. d. Section of vegetative cell, showing pyrenoglobuli scattered throughout the whole pyrenoid.

unicellular and solitary. Cells are spherical or subspherical, 3.0–15 μm in diameter. Chloroplast is parietal and band- or cup-shaped, sometimes with irregularly undulated margin, filling barely about a half of cell periphery, and often removed from the cell wall. The pyrenoid is spherical to ovoid and surrounded by many starch grains. Oil droplets are sometimes present. Numerous vacuoles filling about a half of cell volume are sometimes observed. Cells are capable of producing 2–16 autospores. Reproduction begins with the cleavage of protoplast into various numbers of portions unequal in size. Autospores are spherical to ovoid, 2.5–4.0 × 2.5–6.0 μm in size. The parent cell wall forms an aperture through which the autospores are discharged.

Electron microscopy (Fig. 3. c, d): The cell wall is single and 0.15–0.25 μm in thickness. The chloroplast consists of thylakoid lamellae arranged in several almost parallel rows. The thylakoids are contacted with the surface of the pyrenoid, and traverse a part of the pyrenoid matrix. Small pyrenoglobuli align both sides of the thylakoids. Some cells have numerous relatively large pyrenoglobuli which are scattered throughout the whole of the pyrenoid matrix.

The gross morphology and the method of autospore formation in our alga agree with *Ch. luteoviridis* UTEX 22.

As shown in Fig. 4b, the thylakoids contact with the surface of the pyrenoid and penetrate into a part of pyrenoid matrix, around which many small pyrenoglobuli attach to the surface of penetrating thylakoids in *Ch. luteoviridis* UTEX 22. A similar pattern of thylakoid and the small pyrenoglobuli distribution is also evident in our alga. Ikeda and Takeda (1995) showed that the thylakoids of *Ch. luteoviridis* SAG 211-2b did not penetrate the pyrenoid, but tubelike structure penetrated the matrix of pyrenoid. These features are not distinct in both *Ch. luteoviridis* UTEX 22 and our alga. Some cells in our alga have large and numerous pyrenoglobuli distributed throughout the matrix of pyrenoid. This various arrangement of pyrenoglobuli was observed in only our alga. Several papers have been published on the number or the localization of pyrenoglobuli in relation to aging or the change of environmental condition for certain species of green algae (Brown and Wilson, 1968; Jacobs and Ahmadjian, 1969; Brown et al., 1987; Ascaso et al., 1988). However, the data presented are still not sufficient to conclude whether these features can be useful as taxonomic marker for *Chlorella* species. Taking these circumstances into consideration, we consider our alga as *Ch. luteoviridis*.

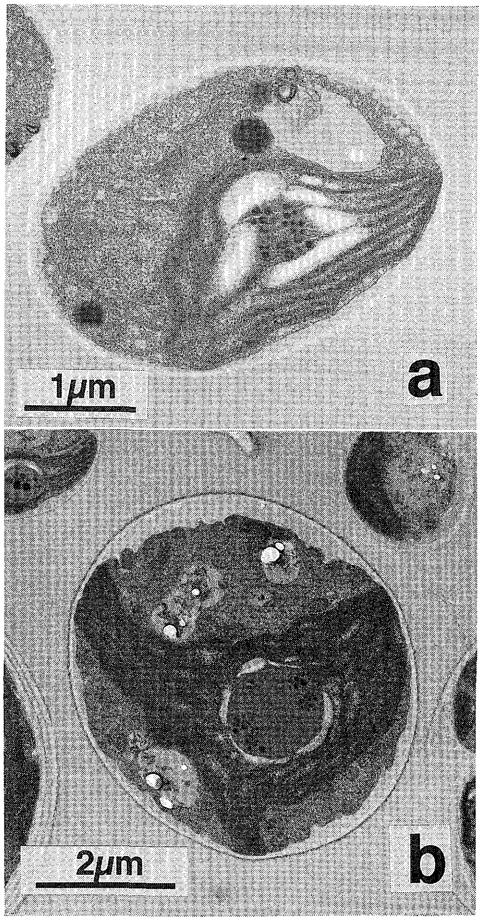

Fig. 4. Transmission electron micrographs. a. *Chlorella ellipsoidea* IAM C-87. b. *Ch. luteoviridis* UTEX 22.

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花方信孝，軽部征夫，千原光雄: **日本産樹皮付着性緑藻 (2)**

内房地区（千葉県）のシュロおよびクロマツの樹皮より単離した *Chlorella* 属の2種について分類学的研究を行った．1種は細胞が長楕円形あるいは楕円形で，残る1種はほぼ球形であった．長楕円形の種は葉緑体が帯状あるいは皿状で，ピレノイドは数個の澱粉粒にかこまれ，小さなピレノグロビュールをもつチラコイド膜が貫通していることから *Chlorella ellipsoidea* Gerneck と同定した．球形の種は葉緑体が帯状で，ピレノイドは多数の澱粉粒にかこまれていた．この種のピレノイド中にはチラコイドが観察されたが貫通しておらず，ピレノグロビュールはチラコイドの両側あるいはピレノイド全体に位置していた．これらの特徴から，これを *Ch. luteoviridis* Chodat と同定した．

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